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RESEARCH ARTICLE

Cytotoxic and anti-metastatic action mediates the anti-proliferative activity of *Rhazya stricta* Decne inducing apoptotic cell death in human cancer cells: Implication in chemopreventive mechanism

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ABSTRACT

Cancer is associated with a high rate of pre-mature mortality worldwide and serves as a significant impediment to extended life expectancy, leading to undesirable long-term effects on the quality of life of the patients. Bioactive chemical products derived from natural sources have an important place in the well-being of the human population since ancient times. Compounds derived from natural sources have dominated the drug discovery programs in the last five decades and comprise a substantial proportion of current-day pharmaceutical agents. *Rhazya stricta*, an evergreen shrub, it belongs to family Apocynaceae of order Gentianales, and the *Rhazya stricta* is known to have certain medicinal properties, as the Middle East and South Asian traditional medicine employed this plant as a cure for different diseases. Herein, the hydro-methanolic extract of *Rhazya stricta* Decne haa been studied for its effect on spectrum of cancer cells, including LNCaP, C4-2B, PC-3 prostate cancer cells, MDA-MB-231, MCF-7, SKBR3 breast cancer cells, A-549 lung cancer cells and BxPC-3, MiaPaCa-2 pancreatic cancer. Hydro-methanolic extract of *Rhazya stricta* Decne in a dose-dependent manner, showed progressive inhibition of cell growth by induction of cell death in the treated human cancer cells. The IC_{50} value range for different breast cancer cell lines was 142-178 μg/ml; for prostate cancer cell lines, it was 90-148 μg/ml; for pancreatic cancer cell lines 116-166 μg/ml and for the lung cancer cells, it was reported to be 180 μg/ml. The anti-metastatic potential of extract was indicated by the notable reduction in invasiveness and cell migration. Moreover, the structurally diverse phytochemical rich extract, also downregulates the signaling of NF-kB and the NF-kB downstream target cytokine VEGF, providing an insight into the anticancer action mechanism.

Keywords: Cell biology; Cancer chemoprevention; Anticancer drugs; Cell signaling; Natural compounds

INTRODUCTION

Cancer is considered to be a multifactorial disease with a high burden of pre-mature mortality worldwide, which is also associated with the poor quality of life and well-being in the affected human population (Bray et al., 2020). According to GLOBOCAN 2020, by the year 2040, an increase of 47% to 28.4 million new cancer cases has been projected (Sung et al., 2021). Such statistics of incidence and the associated mortality of cancer disease warrant stupendous and novel ideas to resolve the existing challenges. Also, sustainable actions that focus on preventive and therapeutic strategies for cancer are also required to be promoted. Several epidemiological studies have indicated the significance of natural products derived from fruits and vegetables in cancer chemoprevention (Grosso et al., 2013; Ullah et al., 2016). Plant phytochemicals are rich in structurally diverse molecules that can effectively inhibit cancer growth and promotion. These plant derived small molecules can inhibit oxidative stress, inflammation. They can induce apoptosis and block cellular oncogenic signaling (Ullah et al., 2014). Studies on experimental

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models and epidemiological investigations suggest that the phytochemicals derived from natural sources have some significance in treatment of diseases. Plant phytochemicals can serve in identifying new molecular entities (NMEs) for drug discovery, and clinical practice (Newman and Cragg, 2020). It is understood that compounds extracted from plants and other natural sources provide a pool of structurally diverse molecules. It has been estimated that around 49% of the approved cancer therapy drugs (from 1940s-2014) have been obtained from natural sources (Newman and Cragg, 2016). *Rhazya stricta* is an evergreen shrub; this plant has been known to have specific medicinal properties. These medicinal properties have been utilized in traditional medicine in South Asia and Middle East to treat various ailments (Gilani et al., 2007). The traditional uses of this plant include disorders such as inflammatory conditions and diabetes (Al-Dabbagh et al., 2018). Studies have also experimentally demonstrated various therapeutic properties of *R. stricta* for insulin insensitivity, multi-drug resistance, cardiovascular diseases and cancer (Baeshen et al., 2015). Although the plant is being investigated for therapeutic properties for long (Mukhopadhyay et al., 1981), recent studies have again highlighted its potential against cancer, while focusing on the mechanisms of chemopreventive actions (Al-Dabbagh et.al.2018; Baeshen et al., 2015). Here, we have described the effects of the hydro-methanolic extract of *R. stricta* based on its potential to interfere with cellular proliferation against a spectrum of cancer cell lines. The cell lines included breast cancer cell line (MDA-MB-231, MCF-7, SKBR3), prostate cancer cell line (LNCaP,C4-2B,PC-3), pancreatic cancer cell line (BxPC-3, MiaPaCa-2) and lung cancer cell line(A-549).

EXPERIMENTAL

Materials, chemicals and reagents

R. stricta aerial parts including stem, leaves and flowers were collected in the spring season from the natural desert habitat of plant in Deesa Valley of Tabuk. Tabuk is north regional province of Saudi Arabia. An expert (Dr. Mohammad Nasir) from our University did the taxonomic authentication of the plant (voucher deposited). Human cancer cell lines were obtained from ATCC (Manassas, VA, USA). Cancer cells PC3, LNCaP and C42B (prostate); MDA-MB-231, MCF-7 and SKBR3 (breast); MiaPaCa-2 and BxPC-3 (pancreatic); and A549 (lung) were used. DMEM (Invitrogen, Carlsbad, CA, USA) was used to maintain cell lines MDA-MB-231, MCF-7, BxPC-3, MiaPaCa-2 and A549 while RPMI 1640 (Invitrogen, Carlsbad, CA, USA) was used to maintain PC3, LNCaP, C42B and SKBR3 cells. Supplementation of cell culture media was done with 10% FBS, penicillin and streptomycin 100U/ml and 10mg/ml respectively. 37^{0}C temperature was set for cell culture and 5% CO_2-humidified condition was provided during the culture experiments. The extract stock solution was made in DMSO. Every time fresh solution were prepared for an assay. Purchase of NF-kB p6 and VEGF assay ELISA kit was done from Abcam, USA and R&D Systems, Inc. USA, respectively. Bayer Pharma, India supplied tannic acid, quercetin and pyrogallol. HPLC grade solvents were purchased from Rankem, India.

Procedure for preparation of hydro-methanolic extract

The following extraction procedure was used with slight modifications in the methodology described earlier by Suarez et. al. (2010). The plant material obtained from aerial parts of *R. stricta* was rinsed immediately with cold water, dried and processed into small pieces and further ground into a fine dry powder. 300 g dry powder obtained from plant material was extracted in 80 % methanol. Mixture was soaked for 24 hour at 40° C in a conical flask in a shaking incubator. After extraction, it was passed through clean cheesecloth that was double-layered. The filtrate was subsequently filtered through Whatman paper (double-layered). To concentrate the filtrate, reduced pressure at temperature 35°C was used in a Buchi rotary evaporator R-210, Flawil, Switzerland. The material was then placed in vacuum at -30° C for three-four days. Vacuum concentration yielded solid/thick pastes. In the end, 28% (w/w) -84 g residual material was obtained as total yield. Material was stored at -35° C for further use.

Analyses of phytochemical constituents

The analytical studies were focused on phenolics, alkaloids, flavonoids, tannins and saponins. Hydro-methanolic extract of *R. stricta* was analyzed for the presence of major classes of phytochemicals. Pyrogallol was used as standard compound for quantitative determination of total phenolic content in the extract (Blainski et al., 2013). Tannic acid as a reference material was used for tannin (Makkar**,** 2000). The flavonoid content determination was made as quercetin equivalents (Miliauskas et. al. 2004). Gravimetric estimation was performed for determination of alkaloids and saponins in the extract. (Harborne, 1986).

Inhibition of cell growth: *In vitro* studies by MTT assay

96-well microtiter culture plates seeded with cells from selected different cancer cell lines were prepared. Culture plates were then incubated overnight. After incubation the culture medium was replaced with a fresh medium. Fresh medium contained vehicle solvent (DMSO) or extract as has been reported for different experiments. Incubation was performed for 4 days. Afterwards MTT solution-25 µl (5 mg/ml in PBS) was introduced into the reaction mixture in each well. After adding MTT plates were incubated for 2 hours at 37° C. After 2 hours, the supernatant was aspirated. MTT test can distinguish metabolically viable cells. MTT

formazan from viable cells was dissolved in 100 µl DMSO and absorbance was monitored at 595 nm using Microplate Reader (TECAN, Durham, NC, USA). Eight replicate wells were used for treatments. Each experiment was repeated at least three times. Moreover, the DMSO present in reaction mixture never exceeded 0.1%.

Apoptotic cell death: Caspase-3/7 Homogeneous assay

Caspase-3/7 homogeneous assay was used for assessing the induction of apoptosis in cancer cells subsequent to the treatment by plant extract (Ahmad et al., 2009). Cells were exposed to different concentrations of extracts or solvent control for 96 hours. After incubation, 100 µl of reagent Apo-ONE1 caspase-3/7 was added to the reaction mixture; samples were properly shaken for 2 min, and then incubated for 120 minutes at RT. Microplate Reader (TECAN) was used to measure the fluorescence. The results were recorded after excitation and emission wavelengths were set at 485 nm and 530 nm respectively.

Soft-agar colony formation assay

Cells ($3x10^4$) from the selected cancer cell lines were seeded in 0.5 ml of culture medium. Culture medium contained media and basal layer of agar 0.7% (weight/volume) (with supplements) along with a top agar layer of 0.3% (weight/volume) in 24-well plates. Extract or the vehicle control were added at the time of seeding the cells. Colonies (>50 cells) were counted after culture time of 22 days. Quadruplicate experiments were performed. The results presented in the manuscript are representative of three independent experimental values.

In vitro cell invasion assay

The in vitro assay for cell invasion was performed on 24-well/8-mm pores transwell permeable supports (Corning, USA) which were coated with growth factor–reduced matrigel. Study was conducted as per the directions of supplier. Initially the trypsinized cells were placed in serum-free medium and then seeded into the transwell inserts. Complete media was present in bottom wells. After 24 hours, cells were stained with cell-permeant dye calcein AM (4 mg/mL) at 37°C for 1 hour. After treatment by enzyme trypsin, the cell detachment from the inserts was obtained. The readings for the fluorescence was measured for cells invading the through matrigel. Arbitrary fluorescence values of control groups without any treatment were set as 1.0. For different treatment conditions relative fluorescence values were calculated.

NF-κB (p65) activation assay

Assay for NF-kB p65 transcription factor is an ELISA-based sensitive method. It is a specific assay for determination of transcription factor-DNA binding activity in nuclear extracts. In the assay method a NF-kB response element sequence within a double stranded DNA is immobilized in a 96-well plate. NF-kB contained in a nuclear extract, binds to the NF-kB response element. The complex is identified using an anti-NF-kB p65 antibody. HRP conjugated secondary antibody provide a colorimetric absorbance at 450nm. The protocol was used as per manufacturer's instructions with our experiment related modification that included induction of NF-κB by prior exposure to PMA (15ng/ml) for 1 hour before the adding of the designated dose of extract (250 µg/mL) to test for measurement of its ability to impede NF-κB activation.

VEGF-ELISA immunoassay

The human VEGF immunoassay is an ELISA-based procedure designed to measure human VEGF in cell culture supernates. Cells were initially seeded in 6-well plates. After overnight seeding, treatment with indicated doses of extract followed, and after another 36 hour, the culture media were collected. Cell debris were removed by centrifugation at 800g for 5 min at 4°C. VEGF assay was performed immediately with supernatant with the use of commercially available ELISA kit supplied by R&D Systems. Concurrently, trypsinized cells in the plate were counted, using hemocytometer.

Statistical analysis

Three different sets of experiments were performed. Each set was measured in triplicate. The outcomes are stated as the mean ± SE. ANOVA statistical analysis was performed. F-test was carried out using SPSS version 11.5 (SPSS, Inc., Chicago, IL). The p values of ≤ 0.05 were considered significant as compared to untreated cells. The p-values are showed in the figures, or in the figure legends.

RESULTS AND DISCUSSION

Diverse phytochemicals are present in hydro-methanolic extract of *R. stricta*

The analysis showed that the extract was rich in certain major classes of phytochemicals, which are predominantly found in various plants and have been reported to exhibit pharmacological properties including chemopreventive action against cancer (Ullah et al., 2014). As reported in Fig. 1, the quantitative estimation exhibited varying concentrations of five kinds of secondary plant metabolites: flavonoids, phenolics, saponins, tannins, and alkaloids. Glycosides, triterpenes, tannins and indole alkaloids are present in the *R. stricta* aerial parts such as leaves and such plant extracts have been reported to exhibit various biological properties. Antimicrobial and anticancer activities are well characterized (Gilani et al., 2007; Baeshen et al., 2009; Marwat et al., 2012). Reports have suggested the presence of more than 100 alkaloids characterized

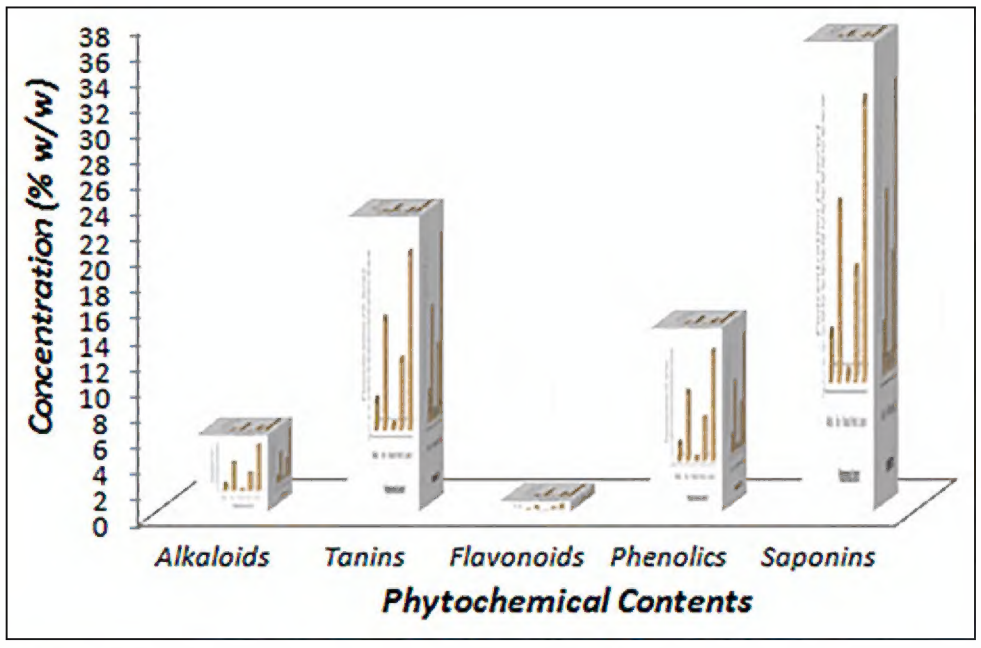

Fig 1. Structurally diverse phytochemicals: phenolics, flavonoids, alkaloids, saponins and tannins as bioactive constituents of *R. stricta*.

from different parts of the plant *R. stricta* since the plant is particularly rich in various alkaloids. Different plant parts including leaves, stems, roots, and legumes are rich in phytochemicals, particularly alkaloids (Gilani et al., 2007). On this account, it can be inferred that alkaloids are amongst the most active components present in high quantities in some medicinal plants. In natural products pharmacologically plant derived alkaloids have shown cytotoxic and anti-metastatic effects when tested on various types of cancers in experimental models. This category of compounds comprise high efficiency drugs such as vinblastine, vincristine, vinorelbine and camptothecin (Lu et al., 2012; Mondal et al., 2019). Interestingly, vinca alkaloids are isolated from *Catharanthus roseus* which as *R. stricta* belongs to Apocynaceae family (Li et al., 2007). Plant derived Tannin extracts induced apoptosis in breast cancer cells and prostate cancer cells (Bawadi et al., 2005). Apoptotic cell deaths *via* activation of caspases 9 and 7 have been reported for tannins such as tannic acid in breast epithelial cells (Booth et al., 2013). In a recent study, hydrolysable tannins induced higher cytotoxicity against human oral carcinoma and salivary gland cancer cells. It was found that such a cytotoxic effect was weaker against normal human gingival fibroblasts. These studies demonstrated the presence of a selective mechanism in such molecules for cancer cells and non-transformed cells (Sakagami et al., 2000). Dimeric compounds were reportedly more cytotoxic than monomeric compounds indicating the implication of structure-activity relationship in cytotoxicity. Studies on saponins which are classified as triterpenoid saponins and steroidal saponins, have also demonstrated significant chemopreventive actions. Various anti-cancer activities include impeding the cell proliferation (Chen et al., 2016), angiogenesis (Zeng et al., 2015), metastasis (Cheng et al., 2016) and promoting MDR-reversal (Zhu et.al.2016). These activities operate through mechanisms of programed cell death and promotion of cell-differentiation (Xu et al., 2016). In therapy of cancer, saponins are prescribed along with immunotoxins. Saponins increase the selectivity of given immunotoxins against cancer cells. It has been noticed that the normal cells remain refractory to the cytotoxic effects. Certain saponins considerably improved the efficacy of chemotherapeutic agents as adjuvants. Various drugs tested in adjuvant therapies include cisplatin, paclitaxel and doxorubicin (Koczurkiewicz et al., 2019). Human epidemiological data have reported protective roles of a large number of plant-derived phytochemicals which are present in diets. Plant derived compounds such as resveratrol (stilbene), genistein (isoflavone) and sulforaphane (isothiocyanate) are active against a variety of cancers as evident from the epidemiological studies (Ullah et al., 2014; Houghton et al., 2019).

Human cancer cells treated with varying concentrations of *R. stricta* extract show dose-dependent inhibition of cellular proliferation

To measure the anti-proliferative potential of the extract, different human cancer cell lines were exposed to the incrementing doses of the extract for 96 hour followed by MTT assay. Cancer cell lines included in the studies were breast (SKBR3, MDA-MB-231, MCF-7), prostate (LNCaP, PC3,C42B), pancreatic (BxPC-3, MiaPaCa-2) and lung cancer cells (A549). The anti-proliferative activity has been shown in the results (Fig. 2). The results indicated that a dose-dependent inhibitory effect was obtained on all cancer cell lines tested with *R. stricta* extract ($p<0.05$). The IC_{50} values (Table 1) showed that the extract exhibited most effective inhibitory effects against prostate and pancreatic cancer cells. The IC_{50} value of 90.1 µg/ml, 108.7 µg/ml, and 116.4 µg/ml were recorded for LNCaP, C4-2B and BxPC-3 cell lines respectively. It also appears from the results that certain concentrations of the extract induced 80-95 % inhibition of cellular growth in specific cancer cell lines. These results on a spectrum of cancer cell lines demonstrate a consistent activity of the extract against cancer cells with origin in different tissues and organ types as used in the current study. Loss of cell viability in a concentration-dependent manner for both aqueous and ethanolic extracts of *R. stricta* was demonstrated by an *in vitro* study. Ethanolic extracts were found to be more active than the aqueous extract when comparative study was performed (Nabih et al., 2012). Recently, studies showed that *R. stricta* significantly enhanced the mortality of cancer cells. Study was performed on human hepatoma cells (HepG2). Study found that the treatment with the lowest concentration of 30 µg/mL exhibited significantly enhanced mortality of cancer cells. (Al-Dabbagh et al., 2018). The cytotoxic effects of the crude alkaloid fraction of the *R. stricta* extract has also been observed in glioblastoma U251 cells. In glioblastoma U251 cells. Effective inhibition of cell viability was obtained in

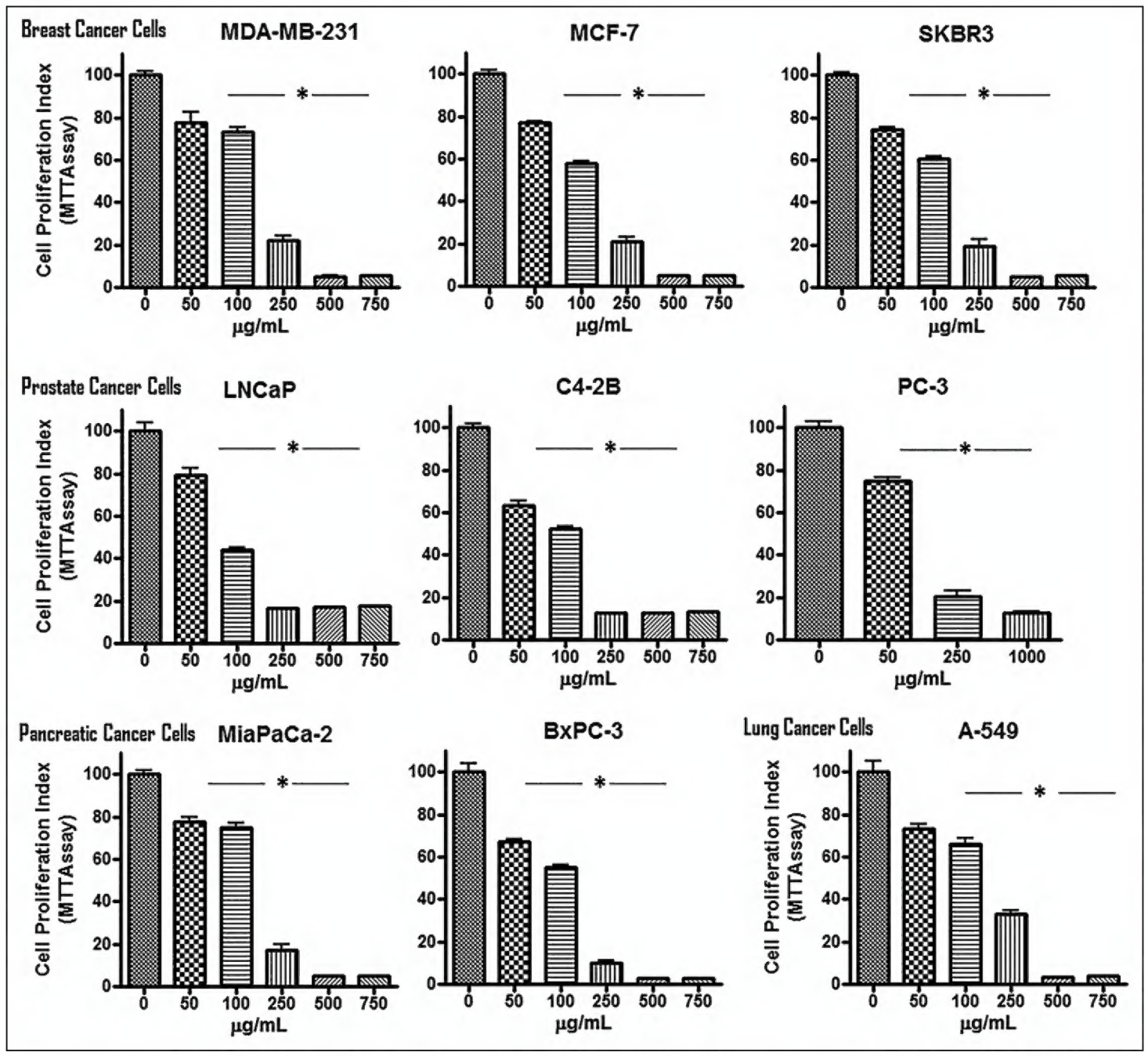

Fig 2. Growth inhibition in cancer cell lines induced by the hydro-methanolic extract of *R. stricta*. **P<0.01/* P<0.05.

Table 1: Inhibitory effect of the hydro-alcoholic extract of *R. stricta* against cancer cells

Cancer cells		IC_{50} µg/ml (96 h)
Breast	MDA-MB-231	178.6±5.9
	MCF-7	142.4±3.6
	SKBR3	148.4±4.1
Prostate	LNCaP	90.1±1.2
	C4-2B	108.7±1.6
	PC3	148.3±3.7
Pancreatic	MiaPaCa-2	166.5±3.3
	BxPC3	116.4±2.0
Lung	A-549	180.1±4.0

dose- and time-dependent manner. The study reported IC_{50} values of 200, 135 and 80 μg/mL at the end of day 1, 2 and 3 of treatment, respectively (Elkady et al., 2014). Interestingly the same study also examined the viability of non-malignant human foreskin fibroblasts (HF-5) cells when treated with the extract; the tested cell line were significantly resistant to the extract induced growth inhibition, This study suggested the selective response of the transformed cells towards the cytotoxic activity of the extract. *R.stricta* extract showed anti-proliferative and growth inhibitory effects on different cancer cells and showed sensitive dose-response curves. These observations have been found consistent for different types of cancer cells, however with varying degrees of responses that differ with dose and time of treatments.

R. stricta extract caused progressive induction of programed cell death (caspase-dependent) in treated cancer cells

Apoptosis is the primary cell death pathway involved in the cytotoxic mechanism of certain anti-cancer drugs. It has been established that exposure to anticancer agents potentially forces the tumor cells to undergo apoptosis (Martin, 2006). Our study evaluated the capability of the extract to effectively activate programed cell death in the cancer cells. The response was characterized by the activation of caspases and their noticeable expression in these cells. As a measure of apoptosis, caspase-3/7 homogeneous assay was utilized which indicates the levels of active caspase-3 and/or caspase-7 by means of fluorescence. The results of caspase-3/7 homogeneous assay are shown in Fig. 3. Plant extract showed dose-dependent progressive induction of apoptosis in different cancer cells. The caspase-dependent apoptosis is indicated by the presence of activated caspases in the plant extract treated cells; caspases are known to be involved at effector stage of apoptosis (Florentin and Arama, 2012). Caspase dependent cell death is the result of interaction of these constituent

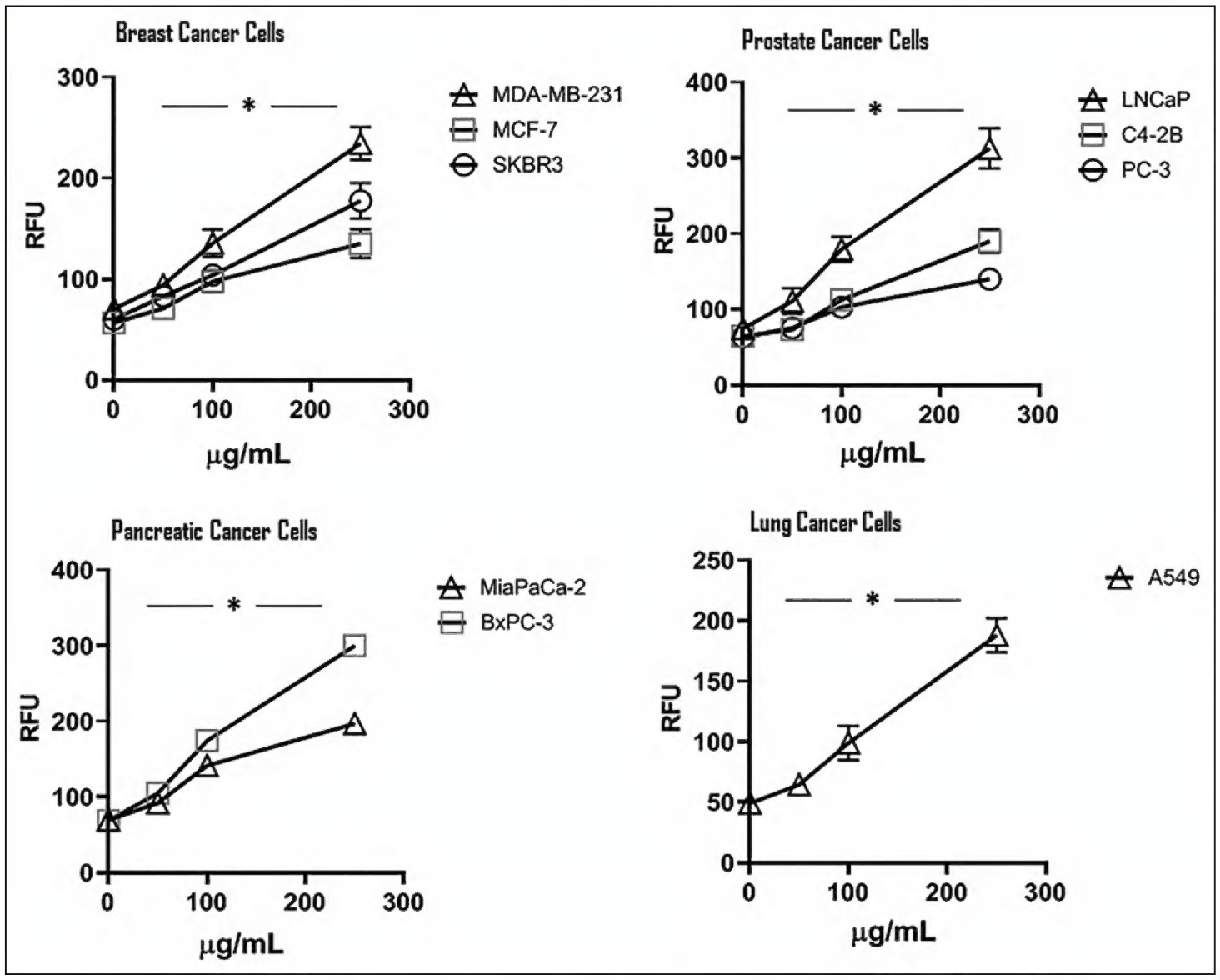

Fig 3. Induction of apoptosis in cancer cells with the reported concentrations of *R.stricta* extract (96 h). *P < 0.05.

caspases and the classified intrinsic-caspases pathways. All these events eventually congregate in the activation of caspase-3 and/or −7. Activation of these caspases results in caspase-dependent cell death in cancer cells (Corbat et al., 2018). A number of studies have described the important role played by natural compounds causing the activation of caspases and thus enhancing the anticancer activity (Cho et al., 2014; Wang et al., 2015). Recently, an isoflavone lupiwighteone from *Glycyrrhiza glabra* (licorice) root has been shown to possess anti-cancer effects for ER-positive and triple-negative breast cancer cells (Won and Seo, 2020). The study reported the anticancer activities wherein the compound induced the caspase-dependent apoptotic cell death. Treatment of cells with the compound promoted the activities of different caspase-3, -7, -8, -9, cleaved-PARP and Bax. It also down-regulated the activities of Bid and Bcl-2 *via* molecular mechanism involving inhibition of PI3K/Akt/mTOR pathway which are implicated in cellular proliferation, metastasis and survival (Badr et. al. 2018). Interestingly, alkaloid rich extract of *R. stricta* has shown not only the activation of programed cell death in cancer cells but it has also demonstrated its ability to suppress the necrotic cell death. These findings suggested the significance of apoptosis as a dominant form of cell death in its cytotoxic action against cancer (Elkady et al., 2014;Galluzzi et al., 2018). Similar observation has been reported for pancreatic cancer cells PANC-1 and AsPC-1, which presented apoptotic characteristics with significant DNA fragmentation on treatment with the crude alkaloid extract of *R. stricta* (Shaer et. al. 2019).

In-vitro inhibitory effect against colonization and invasiveness in cancer cells treated with *R. stricta* extract

Colonization assay measures the ability of cancer cells to survive after treatment with a cytotoxic compound and subsequently grow to form colonies. Such an anchorage-independent clonogenic assay is an effective indicator of the ability of a single cell to form multicellular colony in media. In this assay semi-solid agarose gel suspensions serve as 3-D environment devoid of attachment for cells to clone into a multicellular colony (Horibata et al., 2015). The results from this assay are displayed in Fig. 4A. Results showed that *R. stricta* extract progressively inhibited the colony formation. Colony formation was inhibited for cancer cells derived from multiple cell lines. This inhibition of colony formation occurred in a dose-dependent manner as shown. The observed inhibition reached > 50 % for tested concentrations in certain cell lines demonstrating growth suppressive action of the extract. It is a physiological phenomenon for normal cells to avoid anchorage-independent growth, which otherwise undergo a particular type of cell death, called anoikis (Taddei et al., 2012). Moreover, it is well established that transformed cells grow without adhesion to a substrate and have the ability to grow under such conditions where anchorage is

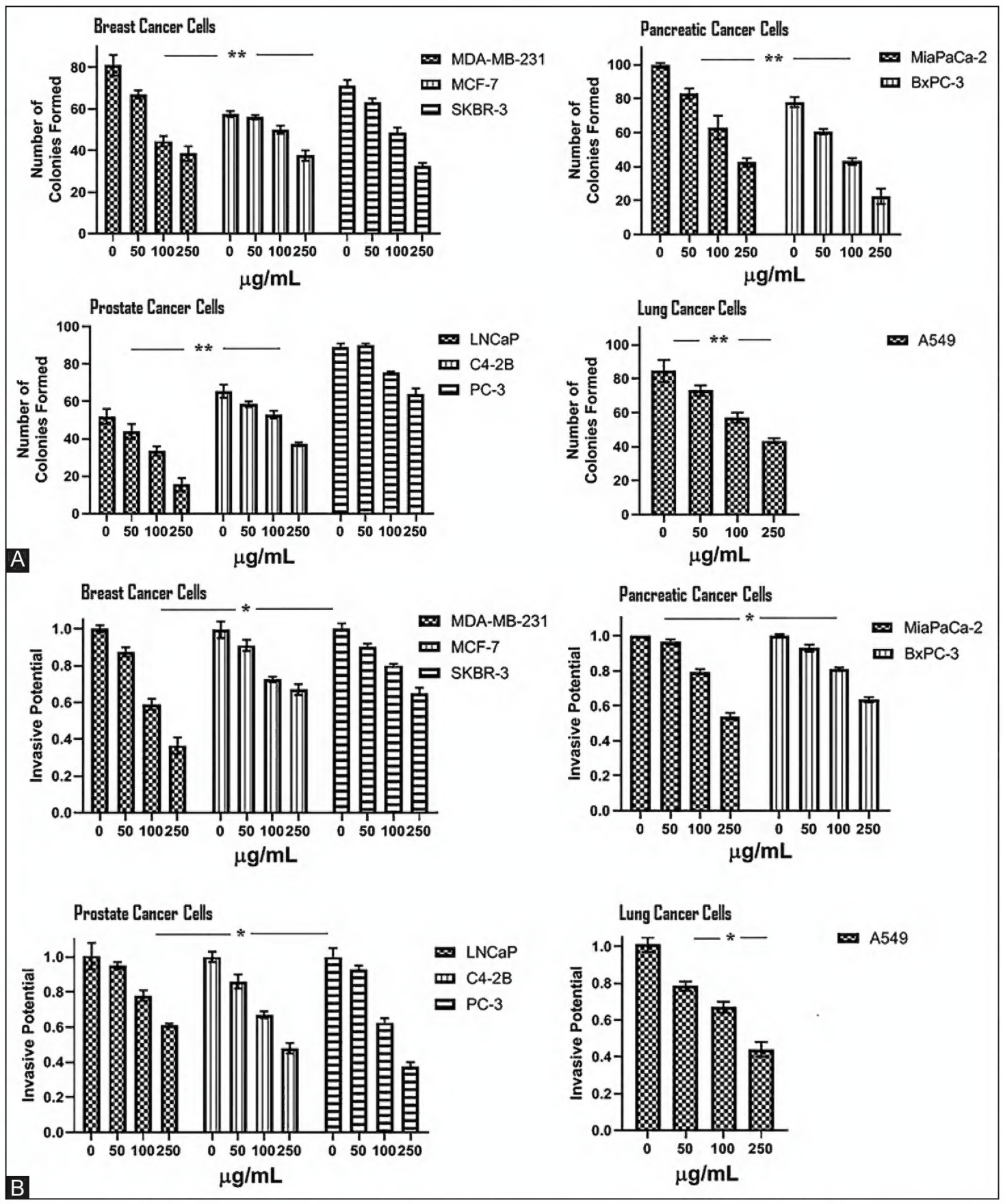

Fig 4. (A) Inhibition of colony formation assessed by soft agar clonogenic assay. Culture was supplemented with indicated doses of *R.stricta* extract and colonies (>50 cells) were counted after appropriate culture time. **P < 0.01. (B): Inhibition of cell migration potential by different doses of *R. stricta* extract in cancer cells. * P<0.05.

not necessary to form colonies, demonstrating clonogenic attributes. Cancer progression often leads to metastatic foci in distant parts of the body following metastasis, which involves mechanisms that grants cancer cells certain invasive characteristics (Fares et al., 2020). In order to further assess the anticancer properties of the extract, invasion assay was performed. Invasion assay measures the invasiveness or metastatic potential of cancer cells. The results are shown as a graph in Fig. 4B. The relative invasive potential was found to decrease for treated cells as compared to the untreated cells. Treated cells display a low penetration and invasiveness. Accordingly, the value of fluorescence measurements from the invading cancer cells reduced significantly in comparison to that of control cells. These observations suggest that the extract impedes the metastatic capability in treated cells. Metastasis is a dominant feature in cancer progression in the advanced stages. Metastasis of tumor cells facilitate dissemination from the primary tumor sites. In metastasis, tumor cells reach the circulatory and lymphatic systems, which help in subsequent invasion across the basement membranes and endothelial walls. This process leads to tumor cells colonization at distant organs (Pijuan et al., 2019). Several studies on new molecular entities derived from natural products have shown promising anti-metastatic activities that affect the key molecular events such as epithelial mesenchymal transition (Chanvorachote et. al. 2016; Yen et. al. 2018). In previous studies, potential anti-metastatic role of *R. stricta* extract on human hepatocellular carcinoma-derived (HepG2) cells and breast cancer

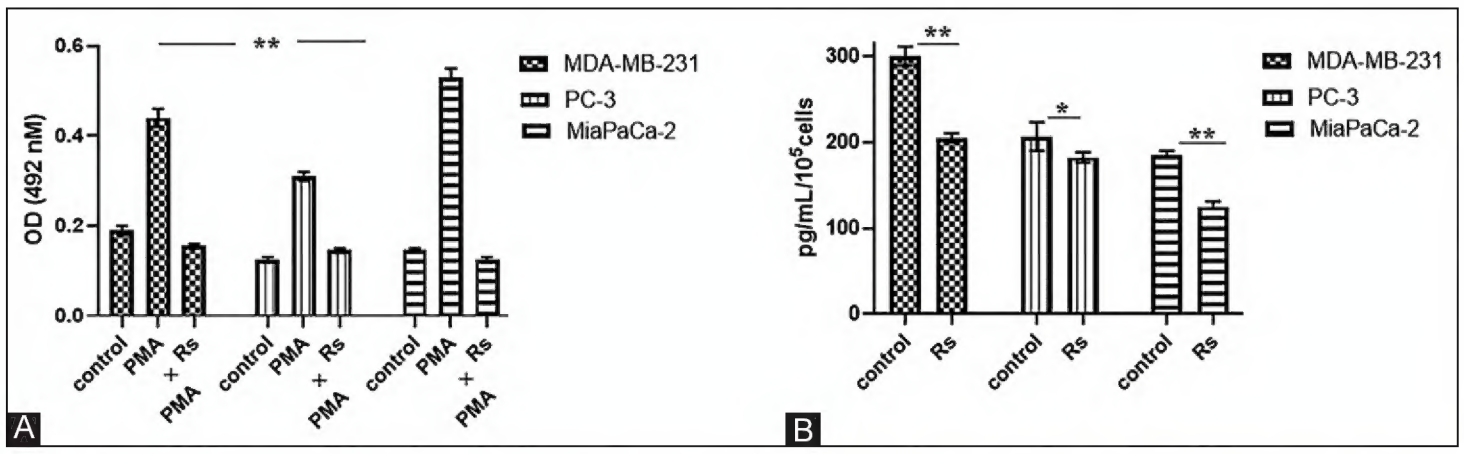

Fig 5. Inhibitory effect of *R. stricta* extract on (A) NF-κB activation and (B) VEGF secretion in selected cancer cell lines. * P<0.05.

(MDA-MB-231) have also been reported using a distinct assay (wound healing assay), which also demonstrated dose-dependent impediment in the potential of the of cells to undergo invasion and migration (Al-Dabbagh et. al. 2018; Al-Zharani et al. 2019).

The mechanism of anticancer action of *R. stricta* extract involves the inactivation of -NF-κB and VEGF

NF-κB is a transcription factor protein complex involved in several cellular responses. NF-κB is known for the transcriptional activation of genes involved in various signaling cascades. Important signaling cascades affected by NF-κB include inflammation, angiogenesis, cell proliferation, suppression of apoptosis and promotion of metastatic potential (Jost and Ruland, 2007). Interfering with NF-κB can cause tumor cells to abate proliferation and therefore it is expected that mediators of NF-κB suppression might have therapeutic potential to inhibit carcinogenesis (Luqman and Pezzuto, 2010). Fig. 5A shows the extract effectively neutralizing the activation of NF-kB in breast, prostate and pancreatic cancer cells. As seen in the figure, the extract in treated cells inhibited NF-kB activation to the levels comparable to control cells. Chemopreventive drugs such as cisplatin, doxorubicin; and natural molecules like curcumin and resveratrol, impede NF-κB activity and support the significance of NF-κB as a functionally relevant target in cancer chemoprevention (Nakanishi and Toi, 2005; Tergaonkar et.al. 2003; Soobrattee et al., 2006). Furthermore, an important component of the metastatic pathway is angiogenesis. In addition to providing oxygen and nutritional support to the growing tumor, these new vessels are also the major way for tumor cells to exit the primary tumor site and enter the circulation promoting metastasis (Zetter**,** 1998). Vascular endothelial growth factor (VEGF) is pro-angiogenic regulator that increasingly respond to angiogenic signaling and facilitates tumor formation and metastasis (Ferrara**,** 2002). In this context, secretion of VEGF in presence of extract in selected cancer cell lines was examined and results are presented in Fig. 5B. It was observed that the extract efficiently downregulated the level of VEGF in the treated cells. Interestingly, VEGF expression is NF-kB dependent. Adenoviral constructs over-expressing the NF-kB endogenous

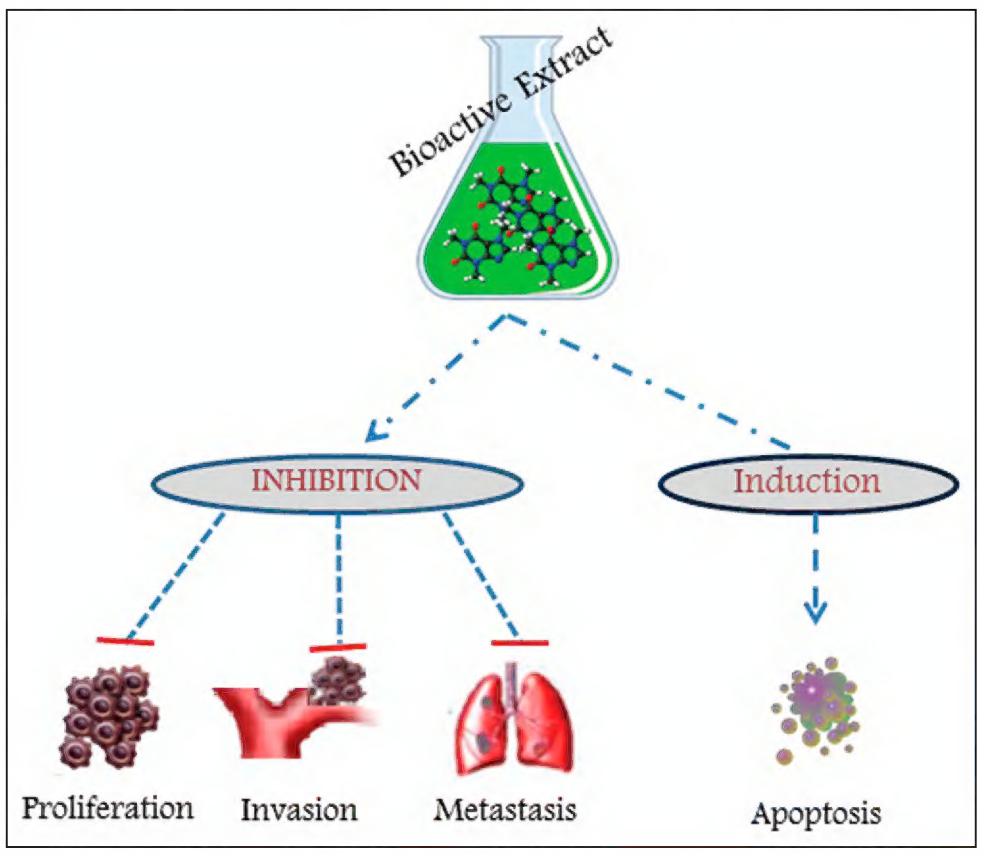

Fig 6. Schematic diagram of the chemopreventive action.

inhibitor (IkappaBalpha) caused inhibition of CD40L-induced expression of VEGF in macrophages when the these were infected with the inhibitor (Kiriakidis et al., 2003). Currently there is an increased interest in acquiring repertoire of drugs which can influence angiogenesis in tumor and impede its enhanced vasculature potential (Bikfalvi and Bicknell, 2002; Harsha et al., 2017). The activation of programed cell death and inhibition of growth mediators like NF-kB and VEGF are important parameters to assess cancer cell response. Present study provides an insight into the mechanistic constrain exhibited by the extract against the oncogenic signaling. Targeting NF-kB activation and VEGF are important in the evaluation of chemopreventive properties of potential anticancer drugs (Wang et.al.2015; Li et al., 2013). A schematic presentation of the chemopreventive action mechanism of such natural products has been shown in Fig. 6.

CONCLUSION

Natural products have a significant place in the well-being of human population since ancient times. Compounds derived from natural sources have dominated the drug

discovery programs worldwide in the last five decades, and comprise a substantial proportion of current-day pharmaceutical agents, most notably as antibiotics and anti-cancer drugs. An array of such compounds with enormously diverse structures has been known to interact with multiple cellular targets and effectively aid in chemoprevention. We have presented herein, an *in vitro* study, which provides a partial evidence of the anti-cancer properties of *R. stricta* and forward a proof-of-concept that might explain the significance of the plant in traditional medicine.

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Statement for conflict of interest

None to be reported.

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